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(54) Title: A METHOD OF ADMINISTERING GENETICALLY ENGINEERED ENDOTHELIAL CELLS TO SITES OF ANGIOGENESIS FOR EFFECTING GENETIC THERAPY

(57) Abstract

A method for administering genetically-engineered endothelial cells to a mammal for effecting genetic therapy, which comprises distally administering to said mammal an effective amount of genetically-engineered endothelial cells, whereby said cells migrate to one or more sites of active angiogenesis in the mammal.

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A Method of Administering Genetically Engineered Endothelial Cells to Sites of Angiogenesis for Effecting Genetic Therapy

Technical Field

The present invention relates to a method of administering genetically engineered endothelial cells to sites of angiogenesis for effecting genetic therapy.

Background Art

Endothelial cells are joined edge to edge in such a manner as to form a membrane of cells. These cells are found on the free surfaces of the serous membranes, as the lining of the heart, blood vessels and lymphatics, the surface of the brain and spinal cord and in the anterior chamber of the eye. Endothelial cells play an essential role in maintaining blood fluidity. See Gray's Anatomy (15th edition, 1977).

At present, it is known that endothelial cells can be genetically modified *in vitro* and then implanted *in vivo* on arterial graft segments or with direct infection of the arterial wall *in vivo* using a replication-defective retroviral vector expressing a recombinant gene. The genes are directly transferred into vascular cells *in vivo* using liposomes. It is also known that liposome-mediated gene transfer *in vivo* can be effected into Kupfer cells in the liver. See Nicolau et al, 1984 Cell Fusion, pp. 254-257.

However, at present, when using genetically-engineered endothelial cells, it is deemed necessary to administer the cells locally so as to directly provide their availability to the site of interest. This has been done either by using genetically-engineered endothelial cells which are immobilized

on a vascular graft or by directly administering the genetically-engineered cells locally onto the wall of a large blood vessel.

However, it would be desirable if reliance upon implanted substrates could be avoided. It would also be desirable if a method of efficient incorporation of genetically-engineered endothelial cells into a microvascular network could be achieved.

Disclosure of the Invention

Accordingly, it is an object of the present invention to provide a means for incorporating genetically engineered endothelial cells into a microvascular network in a stable fashion.

It is also an object of the present invention to provide a method for distally administering genetically-engineered endothelial cells to one or more sites of angiogenesis for genetic therapy.

It is also an object of the present invention to provide such a method whereby it is unnecessary to rely upon implanted substrates carrying the genetically-engineered endothelial cells.

Moreover, it is also an object of the present invention to provide such a method whereby it is unnecessary to directly inject the genetically-engineered endothelial cells to a localized site of interest.

The above objects and others which will become more apparent in view of the following disclosure are provided by a

method of administering genetically-engineered endothelial cells to a mammal for effecting genetic therapy, which comprises distally injecting the genetically modified cells to a mammal, whereby the cells migrate to one or more sites of active angiogenesis.

Brief Description of the Drawings

Figure 1: HUVEC were isolated as described, transduced with the lac Z-containing retroviral vector, enriched by FDG/FACS and stained with X-gal.

Figure 2: RMEC were isolated as described, and subjected to lac Z transduction, FDG/FACS enrichment and X-gal staining.

Figure 3: HUVEC were transduced and enriched as described in Figure 1, then subjected to immunofluorescent staining with anti-human von Willebrand factor antibody. The positive staining confirms that the cells have maintained their endothelial phenotype.

Figure 4: RMEC were transduced and enriched as described in Figure 2 and then incubated with fluorescein-conjugated anti-von Willebrand Factor antibody. These cells also express von Willebrand Factor, as demonstrated by the positive staining.

Figure 5: Irradiated cells secreting FGF were implanted sc and three days later BAG/HUVEC were injected via the tail vein. Ten days later the tissue at the angiogenic implant site was removed and stained with X-gal. The prominent vasculature and vessel tortuosity are in response to the angiogenesis factor. The blue deposits are BAG/HUVEC that were stained with X-gal.

Best Mode for Carrying Out the Invention

In accordance with the present invention, it has been surprisingly discovered that the distal injection of genetically-engineered endothelial cells into a mammal is effective for directing these cells to one or more sites of active angiogenesis. That is, the genetically-modified endothelial cells are now recognized as being capable of migrating through the circulatory system to any one or more sites where active angiogenesis is underway. Sites of new blood vessel formation may include, for example, implants releasing an angiogenesis factor, or tumors, both primary and metastatic, that are engaged in angiogenesis. Once the cells reach the one or more sites they may be stimulated to proliferate along with the endogenous endothelium and eventually become incorporated in the newly formed blood vessel. The engineered cells can then secrete therapeutic agents into the circulatory system for either systemic or local affects.

Tumor-associated angiogenesis is believed to be required by all tumors in order for invasion and metastasis to occur. Weidner, N. et al: Tumor Angiogenesis and Metastasis-Correlation In Invasive Breast Carcinoma, N. Engl. J. Med. (1991): Jan. 3, 324 (1) 1-8. Thus, any metastatic tumor; including for example sarcomas, gliomas and epidermal cancers, such as breast, lung, pancreas, prostate, ovarian, uterine, cervical, gastrointestinal and skin may be used as a target for genetically-modified endothelial cells administered in accordance with the present invention.

Further, Kaposi's sarcoma, a tumor which is characterized by an abnormal endothelial cell proliferation, is associated with the production of a potent angiogenesis factor known as

K-FGF and is particularly amenable to the methods of the present invention.

In general, for the treatment of cancer, it is contemplated that the genetically-modified cells function like a "trojan horse", whereby they first become incorporated within the surrounding tissues or even within the blood vessels induced to form by the tumor. Subsequently, the cells release various molecules that adversely affect the tumor by interfering with its blood supply, by stimulating the immune system to reject the tumor, or by interfering with the processes of tumor growth or invasion. Of course, some substances may act upon the immune response as well as upon the blood supply of the tumor, such as IL-1α, HLA or TNF. Hereinafter, specific subcategories of anti-cancer activity will be considered.

A. Immunomodulation

In particular, IL-2 may be used to recruit cytotoxic T lymphocytes (CTL) and natural killer cells directed against tumor cells.

T-interferon may be used for induction of class II HLA gene expression by tumor cells allowing improved recognition of tumor cells by CTLs.

Also, TNF-α may be used for augmentation of the immune response to tumor cells and damage to endothelial cells supplying the tumor. It is envisioned that the genetically-modified endothelial cells will have become incorporated into the blood vessels supplying the tumor, and, thus, will be optimally situated to deliver the TNF in a locally high concentration to the tumor. This should avoid the toxicities

that have been observed when TNF is administered systemically. Since the endothelial cells producing the TNF are also susceptible to the effects of the molecule, it will be necessary to place the TNF gene under the control of an inducible promoter, such as the metallothionein promoter. TNF expression would be turned on only after the endothelial cells have become incorporated into the blood supply of the tumor.

Furthermore, other cytokines, such as IL-1α, may be used for modulating an improved immune response to the tumor, and are useful in this dry delivery system. However, inducible promoters may be necessary for IL-1α and TGF-β.

B. Compromise of the Tumor Blood Supply

Notably, expression of foreign histocompatibility gene proteins on the surface of the endothelial cells should induce an immune rejection against those cells. The ensuing inflammatory reaction may be able to compromise the blood supply of the tumor and may also improve immune rejection of the tumor.

Also, TGF-β inhibits the growth of certain tumors and may also inhibit the development of the tumor blood supply. This also is a mediator of inflammation.

C. Interference with Tumor Growth, Invasion and Metastasis

Peptides and recombinant antibodies that interfere with growth factor receptors or receptors of molecules involved in tumor cell invasion, e.g., (a) peptide directed at the urine plasminogen activator (uPA) receptor that is expressed by tumor cells and interacts with uPA to activate that molecule. The activated enzyme, in turn, activates other enzymes

involved in tumor invasion; (b) peptide that may interact with receptors of IGF-I and IGF-II, FGFs, or erbB2, a receptor molecule important in breast cancer.

In addition to cancer, various non-neoplastic diseases may also be treated in accordance with the present invention.

For example, hemophilia A and B may be treated with cells expressing Factors VIII and IX.

Additionally, thrombotic disorders may be treated with cells expressing anti-thrombin III, Protein C or Protein S in patients with deficiencies of these proteins.

Further, endocrine deficiencies may be treated with cells producing growth hormone or insulin. However, a better gene regulation is required for the insulin gene because its expression must be linked to the level of glucose in the blood.

Moreover, bone marrow failure states such as aplastic anemia, cyclic neutropenia, bone marrow hypoplasia following chemotherapy or bone marrow transplantation may be treated with cells producing G-CSF, GM-CSF, IL-3, M-CSF or c-kit ligand.

Further, anemia of chronic renal failure may be treated with cells producing erythropoietin.

Furthermore, since sites of wound healing and inflammation are also sites of angiogenesis, the genetically modified endothelial cells can be utilized to target these sites with cytokines or other proteins designed to modulate the inflammatory and wound healing responses. For example,

wound healing may be augmented with the delivery of platelet-derived growth factor (PDGF) or transforming growth factor beta (TGF-β). Where the inflammatory reaction is undesirable, inhibitory peptides can be delivered to those sites.

Additionally, it is also know that tumor cells express cell adhesion molecules (CAM) which enable particular cells to seed particular anatomic sites, i.e., certain tumors metastasize preferentially to lung and not to liver. In order to better target metastases to these sites, endothelial cells can be isolated from either the particular tissue or even from a tumor metastasis for genetic modification. Since these endothelial cells possess the CAM that interact with tumor cells, they may be better able to home those sites when reintroduced back into the individual. Alternatively, endothelial cells can be genetically modified to express specific CAM, in addition to therapeutic molecules, in order to target particular anatomic sites for the treatment of both malignant and non-malignant conditions.

Thus, in accordance with the present invention, any site where active angiogenesis is underway may be used as a target site for the migrating genetically-engineered endothelial cells. Upon reaching the one or more sites where active angiogenesis is occurring, the genetically-engineered endothelial cells eventually become incorporated in the newly formed blood vessel. The present invention is predicated upon the discovery of a remarkable affinity between endothelial cells and sites of active angiogenesis.

As another example, the placenta is also a site of active angiogenesis in pregnancy. Thus, the present invention may be used to direct genetically-engineered endothelial cells to the placenta by distal injection.

When used in conjunction with pregnancy, therapeutic agents may be effectively delivered therefor. For example, endothelial cells may be used which are capable of expressing therapeutic products which minimize or counteract the effects of fetal protein deficiency or genetic defects.

In accordance with the present invention, endothelial cells from any source, such as large or small blood vessels, may be used, conventional techniques may be used for microvascular endothelial cell isolation. See, for example, Folkman J. et al: Long-term culture of capillary endothelial cells. Proc Natl Acad Sci USA 76:5217 (1979); Voyta JC et al: Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein, J Cell Biol. 99:2034 (1984); Zetter B.R: Culture of capillary endothelial cells, in Jaffe E.A. (ed): Biology of Endothelial Cells, Boston, Martinus Nijhoff (1984), p. 14.

In particular, conventional techniques may be used for large vessel and human umbilical vein endothelial isolation. See, for example, Jaffe E.A., Culture and identification of large vessel endothelial cells, pp. 1-13, in Biology of Endothelial Cells, Jaffe, E.A. (ed), Martinus Nijhoff Publishers, Boston (1984).

Furthermore, conventional techniques may be used in the genetic modification and implantation of endothelial cells. See, for example, Zwiebel JA et al: Recombinant gene expression in human umbilical vein endothelial cells transduced by retroviral vectors. Biochem. Biophys. Res. Commun. 170:209 (1990); Zwiebel JA et al: High-level recombinant gene expression in rabbit endothelial cells transduced by retroviral vectors. Science 243:220 (1989); Dichek DA et al: Seeding of intravascular stents with

genetically engineered endothelial cells. Circulation 80:1347 (1989); Nabel EG et al: Site-specific gene expression in vivo by direct gene transfer into the arterial wall. Science 249:1285 (1990); Nabel EG et al: Recombinant gene expression in vivo within endothelial cells of the arterial wall. Science, 244:1342 (1989); and Wilson JM et al: Implantation of vascular grafts lined with genetically modified endothelial cells. Science 244:1344 (1989).

The genetically-engineered endothelial cells of the present invention may be transformed using any conventional methodology to contain donor genes capable of expressing therein to secrete various therapeutic products into the circulatory system.

Although any donor gene may be used which is capable of expressing a product in and secreting it from endothelial cells, mention may be made of several non-limitative examples.

For example, cells in tumor metastasis deposits may secrete cytokines such as IL-1, IL-2, IL-4, TNFα or β, interferon or allogeneic histocompatibility (HLA) antigens to initiate an immune rejection of the tumor, or alternatively secrete agents which would interfere with further angiogenesis, such as growth factor receptor-blocking peptides.

The present invention may also be used to effect systemic secretion of agents, such as Factor VIII or to interfere with the neovascularzation of diabetic retinopathy.

In particular, the following illustrative, but non-limiting examples of donor genes may be mentioned: adenosine deaminase (ADA), Factor IX, hematopoietic growth factors (GM-

CSF, G-CSF, M-CSF, erythropoietin, kit ligand, IL-3), protein C, protein S, hirudin, insulin, growth hormone and parathyroid hormone.

Importantly, and in contrast to conventional methodologies, the present invention does not involve the implantation of cells previously attached to an implantable device that contains an angiogenesis factor, nor does it involve the injection of cells to a localized site of a large vessel wall. In essence, the present invention entails the distal administration of genetically-modified endothelial cells that are capable of migrating throughout the body to microvascular sites of angiogenesis.

Generally, the present invention may be used in the treatment of cancer site-directed anti-tumor therapy. It may also be used in various drug delivery systems in endocrine and vascular diseases. The present invention may also be used in the treatment of diabetic retinopathy.

For example, in the treatment of cancer, it is now possible to deliver cytotoxic molecules locally to specific sites in high concentrations without suffering systemic effects of the cytotoxins.

In general, the endothelial cells in accordance with the present invention may be engineered using a modified replication-defective retroviral vector in a conventional methodology or by using electroporation or liposome-mediated gene transfer. More specifically, the present invention also utilizes the LacZ gene which encodes the enzyme, β-galactosidase. This enzyme is capable of acting upon a number of chromogenic substrates, by which cells may be tracked following implantation into animals. However, while the

methodology of Nabel et al entails introducing cells containing the recombinant gene directly onto a pre-existing arterial wall using a specially designed catheter in order to localize the site of injection, the present invention is radically different as it entails the distal introduction of endothelial cells.

The present method requires the presence of an angiogenesis factor to generate new microvessel formation into which the newly introduced genetically-engineered endothelial cells can become incorporated. This method utilizes non-immortalized, low passage endothelial cells freshly harvested from donor animals and humans.

Thus, the following features, which characterize the present invention, may be noted.

First, in accordance with the present invention, induction of active angiogenesis is concurrent with the introduction of genetically-engineered endothelial cells. The cells may be distally administered intravenously, or intraarterially with or without a catheter. Thus, in using angiogenesis, a site of implantation for the circulating cells is created.

Second, by genetically labeling the endothelial cells with the LacZ gene, the fate of the cells can be monitored in vivo using the conventional method of Sanes. See Sanes JR et al: The Use of Recombinant Retrovirus To Study Post-Implantation Cell Lineage in Mouse Embryos. EMBO 5:3133 (1986). In using this technique, blue staining of cells in the site where angiogenesis occurs is observed. In accordance with the present invention, these cells can be detected for several weeks following the introduction of the endothelial

cells.

It appears that the mechanism for cell implantation may be due to a combination of factors, including (1) a chemo-attractant effect of the angiogenesis inducing factor, (2) a proliferative response of the factor, (3) a survival promoting effect of the factor and (4) a site that is structurally advantageous to the settling of the migrating cells.

In general, any donor genes may be used in accordance with the present invention provided that they are expressible and secretably from endothelial cells containing them, and that they code for a protein product which is therapeutically effective against one or more diseases or conditions.

For example, in the treatment of cancer, donor genes encoding γ-interferon, interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-6 (IL-6), TNF-α, TGF-β, HLA, tissue inhibitor of metalloproteinase (TIMP) I and TIMP II, plasminogen inhibitor-I (PAI-I), tissue plasminogen activator (t-PA) and urokinase (U-PA) may be used.

Generally, the parent endothelial cells used for preparing the transformant cells are obtained from the mammalian body being treated. For humans, it is also possible to use parent endothelial cells from an identical twin. Such cells may be obtained from subcutaneous fat or body tissue.

Further, the transformant cells, regardless of therapeutic utility, are administered in an amount of about 10^6 to 10^{12} cells/kg of body weight. For higher cell numbers, it is preferably to administer the cells by slow infusion.

For example, a cell solution in sterile 5% saline or

dextrose-5%-saline solution may be used having a concentration of about 10^6 cells/250-500 μl. Such a solution can be administered intravenously in about 30 seconds. Thus, larger numbers of cells may be conveniently administered by slow infusion. However, the cell concentration of these solutions may be varied.

The present invention will now be further described by reference to certain examples which are provided solely for purposes of illustration and are not intended to be limitative.

MATERIALS AND METHODS

I. Endothelial Cell Isolation

A) Human umbilical vein endothelial cells (HUVEC).

Freshly obtained human umbilical cords were cannulated and irrigated with warm (37°C) PBS. The umbilical vein was filled with 0.1% Type I collagenase and incubated at 37°C for 10 minutes. The collagenase solution was flushed out of the vein and the cells are washed in M199 with 20% fetal calf serum (FCS), 25 mM HEPES, 2 mM L-glutamine, penicillin (100 units./ml), streptomycin (100 μg/ml), and Amphotericin B (5 μg/ml). The cells are plated in the same medium containing ECGS (50 μg/ml) onto fibronectin-coated (1-1.5 $\mu g/cm^2$) tissue culture dishes.

B) Rat microvascular endothelial cells (RMEC).

Epidydimal fat from young adult rats was dissociated with 0.3% collagenase in isolation medium, (M199) buffered with 0.71 g sodium bicarbonate [$NaHCO_3$]/L and 2.21 g Hepes/L and

supplemented with 0.1% bovine serum albumin (BSA) and 25 mg Soybean trypsin inhibitor pH 7.4, for 30 min at 37°C while shaping at 80 cycles/min. The digest was filtered through a 100 μ Nytex filter and centrifuged at 900 rpm for 10 min at room temperature. The cell pellet was then washed with Ca++, Mg++ -free Hanks balances salt solutions (CMF-HBSS), pH 7.4, and suspended in 14 ml of CMF-HBSS buffered with 0.35 g $NaHCO_3$/L and supplemented with 0.25% BSA and 25 mg STI/L, pH 7.4. Subsequently, the cell suspension was thoroughly mixed with 21 ml iso-osmotic Percoll with 10X CMF-HBSS and centrifuged in a fixed angled rotor (JA-20, Beckman) at 20,000 Xg for 50 min at 4°C. Tufts or capillary endothelial cells from the twelfth to the fourteenth mL fractions (density, 1.048 g/ml) of the gradient are collected, washed, and seeded in fibronectin-coated dishes containing complete growth medium (M199 with 20% fetal calf serum, 2 mM glutamine, 50 μg/ml ECGS and 50 units/ml heparin). After 72h incubation in a humidified incubator of 95% air and 5% CO_2 maintained at 37°C, the contaminating non-endothelial cell colonies are removed by mechanical weeding. The endothelial cells are further enriched by incubation with DiI-AcLDL and subjected to flow cytometry, as described in Voyta et al.

II. Retroviral infection of endothelial cells

Twenty four hours prior to infection, endothelial cells are seeded onto fibronectin-coated 100 mm plastic dishes at a 1:2-1:3 split ratio. To infect the cells, the medium was replaced with 5 ml of amphotropic viral stock containing the BAG retroviral vector (ref) (5 X 10^4 titer) and 8 μg/ml of polybrene. The dishes are returned to the incubator and an additional 5 ml of fresh complete growth medium was added. The dishes are incubated overnight and then refed with fresh complete growth medium. The lacZ-transduced cells are then

selected using FDG-FACS.

III. Selection of cells using flow cytometry (FDG-FACS)

Cells are trypsinized and resuspended in M199 + 5% FCS at a concentration of approximately 10^7/ml. 100 µl of the cell suspension was warmed to 37°C and mixed with 100 µl of pre-warmed (37°C) 2 mM FDG in water. The FDG-cell mixture was incubated at 37°C for exactly one minute and 1.8 ml of ice cold M199 + FCS was added. By incubating the FDG-containing cells on ice for one hour a maximum amount fluorescein is released. The cold temperature "freezes" the cell membranes, trapping both FDG and free fluorescein within the cell. Cells are sorted using a chilled water jacket to prevent leakage of fluorescein from β-gal positive cells. See Nolan GP et al. Fluotescence-activated cell analysis and sorting of viable mammalian cells based on beta-D-galactosidase activity after transduction of Escherichia coli lacZ. Proc. Natl. Acad. Sci. USA 85:2603 (1988).

IV. X-gal staining

Cells: Cells are washed with PBS and fixed for 5 minutes in cold 2% formaldehyde plus 0.2% glutaraldehyde in PBS. Cells are then washed extensively with PBS and incubated at 37°C overnight with 1 mg/ml 4-Cl-5-Br-3-indolyl-β-D-galactopyranoside (X-Gal) in PBS containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM $MgCl_2$.

Figures 1 and 2 illustrate X-gal staining of endothelial cells. In Figure 1, HUVEC were isolated as described, transduced with the lac Z-containing retroviral vector, enriched by FDG/FACS and stained with X-gal. In Figure 2, RMEC were isolated as described and subjected to lac Z

transduction, FDG/FACS enrichment and X-gal staining.

Tissues: Implants of transduced RMEC are removed from animals at varying time intervals (weeks to months following implantation) and processed to detect β galactosidase expression by staining with X-Gal. This simple staining method provides a dramatic visual display of recombinant gene expression *in vivo*. Fixation proceeds for 40-60 minutes. The tissue is washed extensively in PBS and then stained with 1 mg/ml X-Gal in PBS containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM $MgCl_2$. Incubation is performed at 4°C instead of 37°C in order to reduce background staining. Tissues are frozen in OCT compound and sectioned. Cryostat sections are mounted on gelatin-coated slides for microscopic examination. This method provides fine detail of tissue architecture with an appreciation of the β galactosidase-expressing cells in relation to their neighbors in situ.

V. Immunofluorescence for confirmation of endothelial phenotype

Monolayers of BAG-transduced and FACS sorted endothelial cells are examined for the expression of acetylated low density lipoprotein receptor and Factor-VIII related antigen (von Willebrand Factor, vWF) endothelial cell markers.

A) Acetylated low density lipoprotein receptor assay.

Cell cultures for acetylated-low density lipoprotein receptor assay are washed with phosphate buffered saline (PBS) and fed with lipoprotein depleted serum (LDS) for 24h before the assay. Thereafter, the cells are incubated with 10 ug/ml of Dil-Ac-LDL in Medium 199 (M199) containing 20% lipid-

depleted serum (LDS) and incubated for 4h at 37°C. At the end of the incubation period, culture medium was removed and the cells are washed twice with probe-free medium for 5 min. The cells are examined with standard rhodamine excitation/emission filters.

B) vWF assay.

Immunofluorescent staining of endothelial cells for Factor-VII related antigen was performed on cell cultures fixed in 100% methanol for 3 min at -20°C followed by two washes in Hanks Balanced Salt Solution (HBSS). The cells are then incubated in a 1:80 dilution of fluoresceinated goat anti-human Factor-VIII antibody in HBSS for 15 min at room temperature. The cells are washed three times in HBSS over 15 min, mounted in 90% glycerol in PBS, and viewed with Axiophot photomicroscope (Carl Zeiss, INC.) using standard fluorescein filters.

Figures 3 and 4 illustrate the results obtained using the above-described immunofluorescence methods. In Figure 3, HUVEC were transduced and enriched as described in Figure 1, then subjected immunofluorescent staining with anti-human von Willebrand factor antibody. The positive staining confirms that the cells have maintained their endothelial phenotype. In Figure 4, RMEC were transduced and enriched as described in Figure 2 and then incubated with fluorescein-conjugated anti-von Willebrand factor antibody. These cells also express von Willebrand Factor as demonstrated by the positive staining.

VI. Cell implants in nude mice

Induction of neoangiogenesis: implants of FGF-secreting mouse fibroblasts. Nude mice are maintained in a pathogen-

free environment. Five to seven week old female mice are injected subcutaneously along the flank with either 10^7 irradiated or 10^5 non-irradiated NIH 3T3 that had been engineered to secrete FGF (FGF/3T3 cells). See, J. Biol. Chem, Differential Transforming Abilities of Fibroblast Growth Factor-1: Mutagenesis Studies with Signal Peptide and Putative Nuclear Translocation Sequences, Forough R. et al (1991).

Implants of BAG/HUVEC in animals with angiogenic implants. At intervals of several days to weekly, 2-4 X 10^6 BAG transduced HUVEC are injected intravenously via the tail vein into FGF/3T3-bearing animals. Animals are sacrificed and all vital organs, skin, and surrounding tissues undergo X-gal histochemistry.

Following this procedure, blue-staining cells were found at the angiogenic site of animals containing either irradiated or non-irradiated FGF/3T3 implants. An example is illustrated in Figure 5. In Figure 5, irradiated cells secreting FGF were implanted sc and three days later BAG/HUVEC were injected via the tail vein. Ten days later the tissue at the angiogenic implant site was removed and stained with X-gal. The prominent vasculature and vessel tortuosity are in response to the angiogenesis factor. The blue deposits are BAC/HUVEC that ere stained with X-gal.

In accordance with the present invention, any mammal may be treated. For example, the present invention may be used to treat cats, dogs, horses, cows, pigs or sheep. It is particularly advantageous, however, to use the present method to treat humans.

Notably, in accordance with the present invention, in addition to the sites of active angiogenesis described above,

the site or sites of active angiogenesis may be the result of primary or metastatic tumor growth or may be induced by the implantation of an exogenous angiogenesis factor.

Additionally, the present invention may be used in genetic therapy of sites of wound healing. In such a therapy, donor genes which may be advantageously used, for example, are PDGF, bFGF and TGF-β.

Generally, the process parameters of the present invention, such as number of genetically engineered endothelial cells/kg of body weight and concentration of cells in solution administered may be the same as described above regardless of the therapeutic objective. Of course, the duration of treatment will vary as needed with the condition under treatment.

Having described the present invention it will now be apparent to one of ordinary skill in the art that many changes and modifications can be made to the above-described embodiments without departing from the spirit and the scope of the present invention.

Claims

1. A method for administering genetically-engineered endothelial cells to a mammal for effecting genetic therapy, which comprises distally administering to said mammal an effective amount of genetically-engineered endothelial cells, whereby said cells migrate to one or more sites of active angiogenesis in said mammal.

2. The method of Claim 1, wherein said distal administration comprises intravenous administration.

3. The method of Claim 1, wherein said genetically-engineered endothelial cells contain one or more donor genes capable of expressing and secreting therapeutic products in said mammal.

4. The method of Claim 3, wherein said donor genes are selected from the group consisting of adenosine deaminase, Factor IX, Factor VIII, hematopoietic growth factors, erythropoietin, kit ligand, IL-3, protein C, protein S, hirudin, insulin, growth hormone, parathyroid hormone, PAI-I, t-PA, TIMP I and TIMP II.

5. The method of Claim 4, wherein said genetically-engineered endothelial cells contain a gene capable of expressing and secreting Factor VIII.

6. The method of Claim 1, wherein said mammal is a human.

7. A method of effecting site-directed genetic therapy in a mammal, which comprises distally administering genetically-engineered endothelial cells to a mammal in need

of such therapy, in an amount sufficient to effect said therapy, whereby said cells migrate to one or more sites of active angiogenesis, said cells containing one or more donor genes capable of expressing and secreting therapeutic products in said mammal.

8. The method of Claim 7, wherein said distal administration comprises intravenous administration.

9. The method of Claim 7, wherein said donor genes are selected from the group consisting of adenosine deaminase, Factor VIII, Factor IX, hematopoietic growth factors, erythropoietin, kit ligand, IL-3, protein C, protein S, hirudin, insulin, growth hormone and parathyroid hormone.

10. The method of Claim 7, wherein said genetic therapy comprises placental therapy for mammalian fetus.

11. The method of Claim 7, wherein said genetic therapy comprises anti-cancer therapy for sites of primary and metastatic tumors.

12. The method of Claim 11, wherein said metastatic tumors comprise sarcomas, gliomas and epidermal cancers.

13. The method of Claim 12, wherein said epidermal cancers comprise breast, lung, pancreas, prostate, ovarian, uterine, cervical, gastrointestinal and skin cancers.

14. The method of Claim 6, wherein said genetic therapy comprises therapy for sites of wound healing.

15. The method of Claim 11, wherein said donor genes are selected from the group consisting of PDGF, bFGF and TGF-β.

16. The method of Claim 7, wherein said mammal is a human.

17. A method of effecting site-directed genetic, therapy in a mammal for Kaposi's sarcoma, which comprises distally administering genetically-engineered endothelial cells to a mammal in need of such therapy, in an amount sufficient to effect said therapy, whereby said cells migrate to one or more sites of active angiogenesis, said cells containing one or more donor genes capable of expressing and secreting therapeutic products in said mammal.

18. The method of Claim 17, wherein said mammal is a human.

FIG. 1

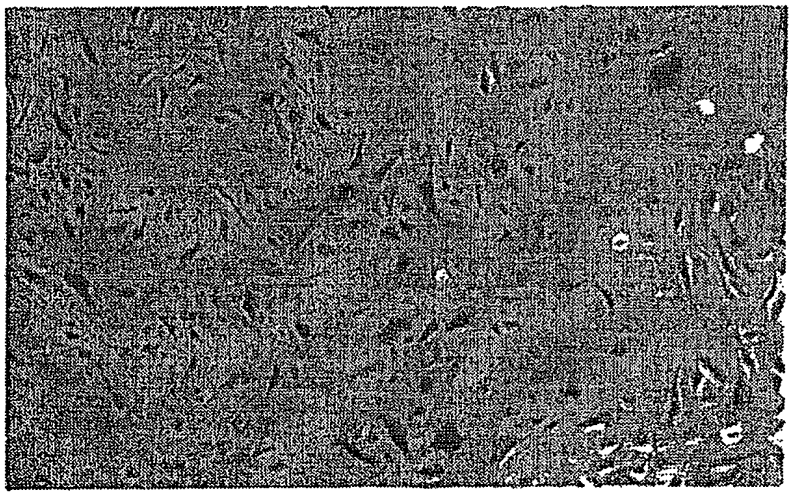

FIG. 2

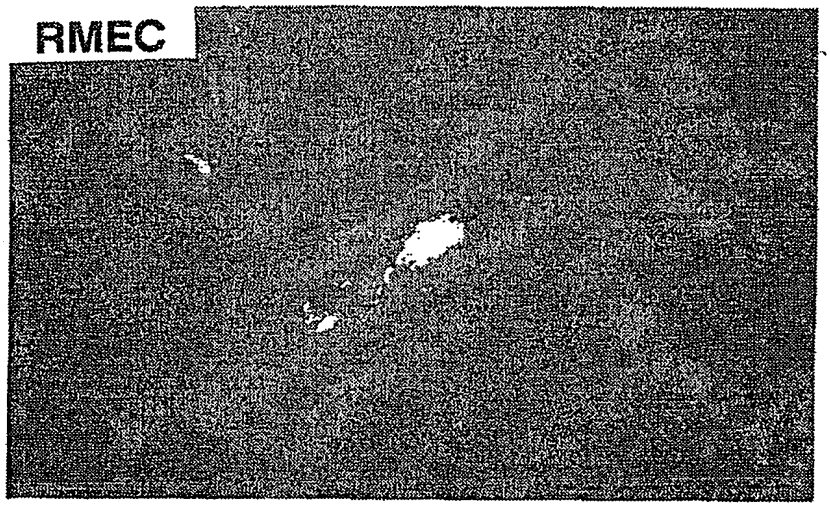

FIG. 3

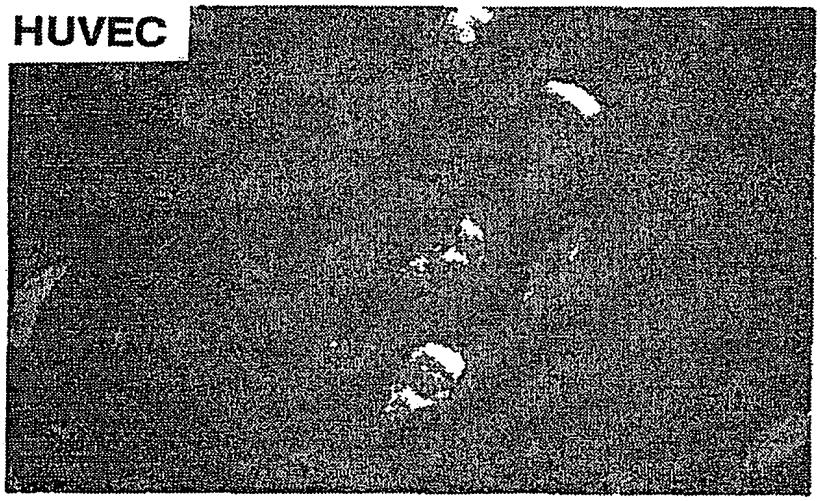

FIG. 4

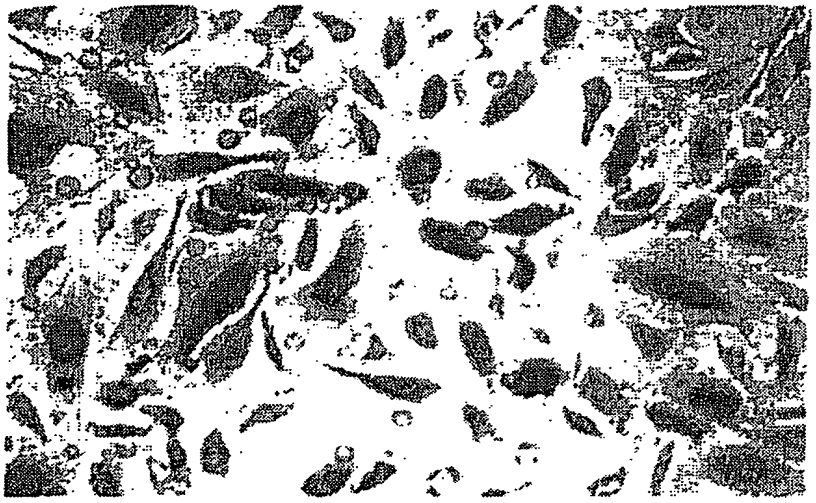

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00007

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :424/93B; 935/62; 435 172.3, 240.2, 69.4, 69.5, 69.6, 69.7, 69.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93B; 935/62; 435 172.3, 240.2, 69.4, 69.5, 69.6, 69.7, 69.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The FASEB Journal, Volume 4, Number 9, issued June 1990, Bernstein et al, " Recombinant gene expression in pulmonary vascular endothelial cells: polarized secretion in vivo", pages 2665-2670, see the entire document.	1-18
Y	PNAS USA, Volume 83, issued April 1986, Moscatelli et al, "Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration", pages 2091-2095, see the entire document.	1-18
Y	WO, A, 90/06997 (Anderson et al) 28 June 1990, see the entire document.	1-18
A	Blood, Volume 77, No. 3, issued 01 February 1991, Dichek et al., "Enhancement of the Fibrinolytic Activity of Sheep Endothelial Cells by Retroviral Vector-Mediated Gene Transfer", pages 533-540, see the entire document, especially page 540.	1-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 March 1993	29 MAR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JACQUELINE STONE Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00007

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Biochemical and Biophysical Research Communications, Vol. 170, No. 1, issued 16 July 1990, Zweibel et al., "Recombinant Gene Expression in Human Umbilical Vein Endothelial Cells Transduced by Retroviral Vectors", pages 209-213, see the entire document.	1-18
A	PNAS USA, Vol. 88, issued September 1991, Yao et al, "Expression of human factor IX in rat capillary endothelial cells: Toward somatic gene therapy for hemophilia B", pages 8101-8105, see the entire document.	1-18
A	Science, Volume 243, issued 13 January 1989, Zweibel et al, "High-Level Recombinant Gene Expression in Rabbit Endothelial Cells Transduced by Retroviral Vectors", pages 220-222, see the entire document.	1-18
A	PNAS USA, Vol. 86, issued October 1989, Thompson et al, "Heparin-binding growth factor 1 induces the formation of organoid neovascular structures in vivo", pages 7928-7932, see the entire document.	1-18
A	Science, volume 244, issued 16 June 1989, Nabel et al, "Recombinant Gene Expression in Vivo Within Endothelial Cells of the Arterial Wall", pages 1342-1344, see the entire document.	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00007

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 48/00; C12N 15/12, 15/15, 15/17, 15/18, 15/19, 15/24, 15/52, 15/58